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INOCULATION OF SOIL

WITH

NITROGEN-FIXING BACTERIA.

BY

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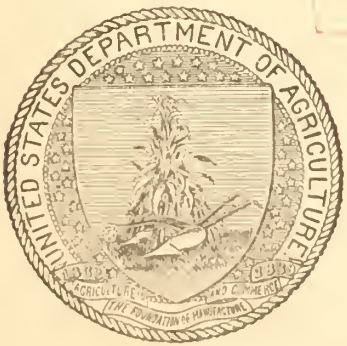

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INOCULATION OF SOIL WITH NITROGEN-FIXING BACTERIA.

INTRODUCTION.

The publication of the results obtained with pure cultures in inoculating leguminous plants has resulted in a very great demand being made upon the Department of Agriculture for inoculating material. The distribution made during 1904 was for the purpose of obtaining a large number of tests of the method under average farm conditions, and it was impossible to anticipate the demand which has arisen this spring (1905), the total quantity prepared for spring distribution having been promised early in February. It is expected, however, that this fall and next spring a further distribution will be made as far as our limited facilities will permit. Statements to the effect that the Department has stopped the distribution of these cultures are therefore erroneous. Applications for future distributions should state what legume is to be sown, time of sowing, and quantity of seed to be treated.

THE COMMERCIAL PRODUCTION OF CULTURES.

The patent which the Department of Agriculture holds upon the method of growing and distributing these organisms was taken out in such a way that no one can maintain a monopoly of the manufacture of such cultures. It is held in the name of Dr. George T. Moore, who developed and perfected the method, as described in former publications. Upon application the Department furnishes without discrimination all necessary information, and as far as possible "starting" or foundation cultures, to the bacteriologists representing experiment stations and commercial concerns which claim to be properly equipped, but it does not in any way guarantee their product. It is not likely that persons without expert knowledge can successfully multiply cultures of these organisms for sale or distribution, and it is understood that any cultures furnished are to be treated according to the methods devised by the Department.

Before experimenting with any bacterial preparations for legumes, the farmer should study thoroughly the soil conditions under which the use of cultures offers any possibility of gain.[a]

Briefly, these conditions may be summed up as follows:

WHEN INOCULATION IS NECESSARY.

Inoculation is necessary—

(1) On a soil low in organic matter that has not previously borne leguminous crops.

(2) If the legumes previously grown on the same land were devoid of nodules, or "nitrogen knots," showing the need for supplying the nodule-forming bacteria.

(3) When the legume to be sown belongs to a species not closely related to one previously grown on the same soil. For instance, soil in which red clover forms nodules will often fail to produce nodules on alfalfa when sown with alfalfa for the first time.

WHEN INOCULATION MAY PROVE ADVANTAGEOUS.

Inoculation may prove advantageous—

(1) When the soil produces a sickly growth of legumes, even though their roots show some nodules.

If the cultures introduced are of the highest virility, their use will often result in a more vigorous growth.

(2) When a leguminous crop already sown has made a stand, but gives evidence of failing, due to the absence of root nodules.

The use of the culture liquid as a spray or by mixture with soil and top-dressing may save the stand if other conditions are favorable.

WHEN INOCULATION IS UNNECESSARY.

On the other hand, *inoculation is unnecessary and offers little prospect of gain*—

(1) Where the leguminous crops usually grown are producing up to the average and the roots show nodules in normal abundance.

Cultures of nitrogen-fixing bacteria are not to be regarded in the light of fertilizers, increasing yields under all average conditions. They do not contain the nitrogen itself, but the bacteria make it possible for the legumes to secure nitrogen from the air (through the formation of root nodules), and where the soil is already adequately supplied with these bacteria it will not usually pay to practice any form of artificial inoculation.

(2) When the soil is already rich in nitrogen.

It is neither necessary nor profitable to inoculate a soil rich in nitrogen when sowing legumes. Not only does the available nitrogen in

[a] Fully described in Farmers' Bulletin No. 214 of the Department of Agriculture, which will be sent without cost upon application to the Secretary of Agriculture.

the soil render the formation of nodules less necessary, but nitrogenous materials in the soil largely prevent the bacteria from forming nodules.

Any increased virility in nitrogen-fixing power possessed by any types of bacteria yet distributed may be rapidly lost in a soil containing an abundance of nitrogen, because the bacteria are rapidly multiplying in a medium in which there is no premium on vigor in securing atmospheric nitrogen.

WHEN FAILURE IS TO BE EXPECTED.

Inoculation will fail where other conditions (aside from the need of bacteria) are not taken into account, as the following:

(1) In soil that is acid and in need of lime.

Liming to correct acidity is as important for the proper activity of the bacteria as for the growth of the plants.

(2) In soil that responds in a marked way to fertilizers, such as potash, phosphoric acid, or lime.

The activity of the bacteria in securing nitrogen from the air and rendering it available to the legumes does not do away with the need for such fertilizing elements as potash and phosphorus.

(3) It must also be remembered that *inoculation does not "act like magic;"* it will not overcome results due to bad seed, improper preparation and cultivation of ground, and decidedly adverse conditions of weather or climate.

In the use of cultures, also, failure is almost certain where the directions are not carefully studied and intelligently followed.

(4) As the physics, the chemistry, and the biology of soils are studied in the laboratory and by means of actual field-plot trials to determine yield and quality of crops and the effect of one crop on the following crops, the very great complexity of soil and farm management becomes more manifest.

The value of pure-bred bacteria, whether associated with the crop or existing independently in the soil, as is true of fertilizers, can not be predicted with certainty on any soil without trial. Success on similar near-by lands may be taken as good evidence. But, unlike fertilizers, bacteria should in time be so inexpensive that each farmer can afford to try them for each leguminous crop on each field or soil type on his farm. The methods of distributing in dried form and the easy methods of multiplying on the farm in sufficient quantities to inoculate fields will make it possible to have all fields inoculated at all times.

COST OF CULTURES.

The question of the proper price for the commercial product is causing considerable inquiry among prospective experimenters and is of importance. The expenses which a commercial concern must necessarily meet, such as rent, heat, light, insurance, postage, advertising,

etc., aside from laboratory assistance and clerical hire, make any comparison with the cost to the Government of similar cultures difficult. The statement that the cultures cost but a few cents an acre refers only to the raw materials which make up the package. It is more than probable that natural competition will considerably reduce the present valuation of the commercial product, and the wisdom of patenting the Department's methods to prevent the formation of a monopoly is already demonstrated.

INCREASING CULTURES.

We are receiving numerous requests from persons who have secured commercial cultures, as well as those sent out from the Department of Agriculture, for information as to the methods employed in producing a large quantity of liquid culture from the dry culture secured as a starter; that is, how to make an "acre culture" do for 25 or 100 acres. Such methods will give good results only when special precautions are taken, and on this account have not been generally recommended. The contaminations, such as yeasts, molds, etc., which are bound to occur to a greater or less extent, are apt to take possession of the culture solution in which the bacteria are being multiplied, and unless great care is taken in thoroughly sterilizing all utensils employed the resulting culture will have no beneficial effect. The extra time required to secure sufficient growth of bacteria in 10 gallons of solution from a dry culture originally intended to produce a 1-gallon liquid culture makes the risk from contamination much greater than where the dry culture is proportioned in size to the larger amount of solution. If a growth sufficient to cloud the solution takes place within two days, the chances of securing an efficient culture are much better than where a longer time is taken; so that the volume of solution prepared should never exceed the actual requirements of the occasion.

The following directions are based on making 10 gallons of liquid culture, sufficient to inoculate 20 bushels of seed. By a little computation the directions may be adapted to 5 gallons or to any intermediate quantities.

PREPARING AND USING THE CULTURE SOLUTION.

To prepare the culture solution, first select the tub, bucket, or other vessel in which you wish to grow the bacteria. *Clean and scald it out thoroughly.* For making the culture solution, rain water that has been thoroughly boiled and allowed to cool is best, though any good drinking water will answer. Add to 10 gallons of water 12 ounces of either brown or granulated (preferably granulated) sugar, 1½ ounces of potassium phosphate (monobasic), which can be obtained at any drug store, and one-sixteenth ounce (30 grains) of magnesium sulphate.

Stir until dissolved, then carefully open the small package containing the bacteria-laden cotton and drop the cotton into the solution. Do not handle any more than is absolutely necessary. Cover the tub with a moist, clean cloth to protect from dust, mold spores, etc. Keep in a warm place, but never let the temperature rise above blood heat. After twenty-four hours add 6 ounces of ammonium phosphate and allow the mixture to stand for another twenty-four hours. The liquid should now be cloudy and ready for use; if sufficient growth has not taken place to bring about this cloudiness, further time should be given, not to exceed a few days.

To inoculate seed.—Use enough culture liquid to moisten the seed thoroughly—about one-half of a gallon per bushel. This inoculating may be done either in a tub or trough, or by sprinkling the culture liquid on the seed on a clean floor and stirring and turning the heaps of seed with shovels until all are thoroughly moistened. After inoculation the seed should be spread out in a clean, shady place until sufficiently dry to handle. If planting is not to be done at once, the seed must be thoroughly dried to prevent molding. In dry weather about 25 bushels can be dried in half a day on 300 square feet of floor space. To do this there must be several open windows or doors to allow a free circulation of air, and the seed must be frequently stirred with a lawn rake. The inoculated seed, if thoroughly dried, may usually be kept without deterioration for several months.

To inoculate soil.—Take enough dry earth or sand so that the solution will merely moisten it. The soil should be preferably from the field to be inoculated, so as to avoid spreading diseases or weeds. Mix thoroughly, so that all the particles of soil are moistened. Thoroughly mix this earth with four or five times as much; spread this inoculated soil thinly and evenly over the prepared ground exactly as if spreading fertilizer. The inoculated soil should be harrowed in immediately to protect the bacteria from sunlight. In using this method allow 1 gallon of the liquid culture to 4 acres or less.

Either of the methods described may be used, as may be most convenient.

To prevent any possible delay, the necessary chemicals should be ordered in advance. If the local druggist does not have them in stock, he can doubtless secure them within a reasonable time.

KEEPING CULTURES FOR FUTURE USE.

The question is frequently arising as to the possibility of the farmer's keeping over cultures from one year to another by soaking up a little of the liquid culture in cotton and drying this cotton. *This proposed practice is not to be advised in any case.* Contaminations take place so readily, and once started spread so rapidly, that for assured

good results it is absolutely necessary to start with a pure culture. The pure culture, moreover, can only be prepared by a trained bacteriologist with laboratory facilities. These cultures in the dry state will keep, under ordinary conditions, from six months to a year.

There is an additional reason, fully as important, which makes the above method impracticable. The cultivation of the bacteria for any considerable length of time in solutions containing ammonium salts rapidly lessens their infective power and their ability to gather nitrogen from the air, so that transfers or new cultures made with absorbent cotton from the cultures prepared for field use would contain organisms of reduced efficiency. It is partly owing to these factors that it is impracticable to distribute the bacteria in liquid cultures and maintain the requisite effectiveness.

In the use of cultures for inoculating soil the farmer should be guided, as in all other matters pertaining to soil treatment, by his own peculiar needs and should not give too great weight to the experiences of others whose soil conditions may differ widely. *It would be unwise to invest largely in any new method for increasing plant growth, whether bacterial or of any other nature, without previously experimenting in a small way.*

DANGER OF INOCULATION BY SOIL TRANSFER.

Satisfactory inoculations have been obtained by transferring soil from old fields on which the legume has been grown, but experience has shown that there are dangers incident to such methods of soil transfer which it is wise to avoid.

The source of supply of such soil should be very definitely known, and in no case should soil be used from fields which have previously borne any crops affected with a fungous disease, a bacterial disease, or with nematodes. Where a rotation of crops is practiced, it is often difficult to make sure of this factor, so that the method of soil transfer is, under average circumstances, open to suspicion, if not to positive objection. Numerous animal and plant parasites live in the soil for years, and are already established in so many localities that it is manifestly unwise to ship soil indiscriminately from one portion of the country to another.

The bacterial diseases of the tomato, potato, and eggplant, and the club-root, brown-rot, and wilt disease of the cabbage, all more or less widely distributed, are readily transmitted in the soil; while in the South and West there are the wilt diseases of cotton, melons, sweet potatoes, cowpeas, and flax, and various nematoid and root-rot diseases which might easily become a serious menace over areas much larger than they now occupy if deliberately spread by the careless use of soil for inoculation purposes. There are several insect and fungous diseases of clover to be avoided, and various diseases of beans and peas. There is also a disease of alfalfa, the "leaf spot," which is

causing damage in some regions. These are only a few of many diseases liable to be transmitted in soils. The farmer should therefore be on his guard. The danger from such sources is by no means imaginary. The Department of Agriculture has had specific cases of such accidental distribution reported, and if the business of selling soil for inoculation is made to flourish by farmers purchasing without question "alfalfa soil," "cowpea soil," etc., there is every reason to believe that experience will demonstrate the folly of such haphazard methods.

Of scarcely less importance is the danger of disseminating noxious weeds and insect pests through this plan of inoculation by means of soils. Even though weeds may not have been serious in the first field, the great numbers of dormant seeds requiring but a slight change in surroundings to produce germination are always a menace. The enormous damage to crops caused by introduced insects and weeds should convey a warning and lead to caution. It is not the part of good judgment to view the risk as a slight one justified by the end in view.

PURE-CULTURE INOCULATION.

The extensive experiments carried on by the Department of Agriculture during 1904 demonstrated the fact that, by the proper use of pure cultures, the nodule bacteria are actually carried into the soil in such a way as to form root nodules, and where other conditions are favorable the inoculation thus brought about makes possible the growth of each legume in soils where it had previously failed from the lack of bacteria. The original cultures used, however, must be prepared with the utmost care and with a view to preserving and increasing their natural power as "nitrogen fixers" rather than merely to make them grow under favorable conditions. The methods devised in our Laboratory of Plant Physiology are based on well-recognized principles of plant breeding and selection, and mark a decided advance in the production of cultures for soil inoculation. The old pure-culture methods were not effective, for reasons clearly stated by Dr. Moore in Bulletin No. 71 of the Bureau of Plant Industry and by Dr. Moore and Mr. Robinson in Farmers' Bulletin No. 214.

The Department of Agriculture is continuing the work of developing types of the bacteria associated with leguminous plants, which will have greater activity, collecting from the air more nitrogen per acre than forms now common in nature or available from laboratories. It is desirable that similar investigations should be conducted with reference to the nitrogen-fixing bacteria existing in the soil independent of the legumes. Important steps have already been taken along this line, but the very large demand for cultures for leguminous crops, by consuming the time of the laboratory force, has seriously retarded these investigations during the past year.

The Department is ready to cooperate with experiment stations and commercial firms, to give and to receive suggestions, to test the product of others, and to furnish, as far as possible, cultures to be tested in the laboratory and under field conditions.

There is nothing in the nature of the processes involved which would prevent a competent bacteriologist, after some experience in this particular field, from producing cultures of as high a grade as those sent out by the Department, and every assistance will be given to competent persons desiring to undertake the work.

A. F. Woods,
Acting Chief, Bureau of Plant Industry.

Approved:
James Wilson,
Secretary of Agriculture.

Washington, D. C., *May 6, 1905.*

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